Department of Health and Human Services
Substance Abuse and Mental Health Services Administration
Center for Substance Abuse Prevention
Division of Workplace Programs

National Laboratory Certification Program (NLCP) **Program Document #37**

Date: July 28, 1999

NOTICE TO HHS CERTIFIED LABORATORIES AND INSPECTORS

Subject: Specimen Validity Testing

The Department of Health and Human Services' (HHS) Mandatory Guidelines for Federal Workplace Drug Testing Programs require laboratories to test urine specimens for only those drugs included in agency drug-free workplace plans. Additionally, the Guidelines permit laboratories to conduct other tests to determine the validity of the specimen.

Certified laboratories reported that the number of adulterated, substituted, and diluted specimens have been increasing. HHS and the Department of Transportation (DOT) began a process utilizing the HHS Substance Abuse and Mental Health Services Administration's Drug Testing Advisory Board (DTAB) to establish a policy for testing, reporting, and interpreting validity test results for specimens tested under federally regulated programs. A team of program staff and consultants determined the normal ranges for the routine clinical measurements that could be conducted on urine specimens and selected levels that were outside the normal range for each clinical measurement. As a result of this effort, National Laboratory Certification Program (NLCP) Program Document #35 was issued on September 28, 1998, to provide guidance to laboratories in determining the validity of urine specimens.

General Guidance/Criteria

Specimen validity testing is the evaluation of the specimen to determine if it is consistent with normal human urine. Validity testing is used to determine if adulterants or foreign substances were added to the urine specimen or if the specimen was substituted. Specimen validity can be determined by establishing parameters that are consistent with normal human urine and/or by testing for the presence of an abnormal or foreign substance in the urine. Specimen validity testing may be conducted on Bottle A and **must** be conducted on Bottle B if Bottle B fails to reconfirm for the requested drug/analyte. Specimen validity tests **may** include, but are not limited to, tests for creatinine concentration, specific gravity, pH, nitrite concentration, pyridine, glutaraldehyde, bleach, and soap. These tests must be performed using methods that are validated by the laboratory.

Specimen validity testing shall be conducted utilizing the following criteria:

1. For dilute specimens, at a minimum, creatinine and specific gravity must be measured by quantitative procedures at a cutoff of 20 mg/dL and 1.003, respectively.

2. For substituted specimens, at a minimum, creatinine must be measured by at least one quantitative procedure on two different aliquots both utilizing the specified cutoff of 5 mg/dL. At a minimum, specific gravity must be performed on one of these aliquots utilizing the specified cutoffs of 1.001 or 1.020.

3. For adulterated specimens, concerning pH and nitrites, at a minimum, two procedures must be performed for pH and nitrites. One procedure must be quantitative and utilize the specified cutoff. The second procedure may be qualitative, must be at least as sensitive as the quantitative procedure, and must be performed on a separate aliquot.

4. For adulterant analytes without a specified cutoff (e.g., glutaraldehyde, bleach, surfactant), at least one procedure must be performed on two separate aliquots.

5. All specimen validity testing methods must be characterized by demonstrating precision and accuracy. Where cutoffs are specified, the limit of quantitation (LOQ) and linearity must be determined. The limit of detection (LOD) must be experimentally determined for qualitative methods.

6. All methods used to characterize and validate these tests must be documented in the laboratory's SOP.

Specific Issues/Comments

Issue 1: Is a certified laboratory required to implement validity testing?

Comment: Currently, validity testing is optional. If a laboratory chooses to conduct validity tests, the laboratory must use the definitions provided in PD35 to report results for specimens that are dilute/adulterated/substituted.

Issue 2: A laboratory may send a specimen to another HHS certified laboratory that has the capability of identifying the presence of an interfering substance/adulterant. Does the laboratory send an aliquot or the entire specimen to the other certified laboratory?

Comment: If a certified laboratory suspects the presence of an interfering substance/adulterant that it is unable to identify and decides to send the specimen to another laboratory, it must send the entire specimen to the other certified laboratory. The selection of the other laboratory must be made in coordination with the MRO. When transferring a single specimen bottle/split

specimen bottles to another certified laboratory, the single specimen/primary (Bottle A) specimen must be resealed. All specimen bottles and chain of custody forms received from the collection site must accompany the specimen bottle(s) to the other laboratory (i.e., copies 1, 2, and 3 of the CCF and all internal chain of custody documents). The primary laboratory should retain copies of all original documents sent to the second laboratory. When the transfer occurs, the primary laboratory must not report any result to the Medical Review Officer (MRO).

Note: The process of transferring specimens to another laboratory may add several days to the reporting time. It is strongly recommended that specimens be kept refrigerated during the transfer to the other laboratory to minimize degradation or changes caused by any adulterants or interfering substances.

Issue 3: When a specimen is sent to a second laboratory, what results does the second laboratory report to the MRO?

Comment: The second laboratory reports results of its drug testing and/or validity testing to the MRO in accordance with PD35. The original laboratory must not report any results to the MRO.

Issue 4: Is a certified laboratory required to accept and test specimens sent to it by another laboratory without prior notification?

Comment: No. Although the NLCP requires every certified laboratory to have the capability to perform reconfirmation testing, a certified laboratory is not required to accept specimens for reconfirmation testing or to accept specimens for validity testing unless this has been agreed upon before the specimens are sent by the first laboratory. Each laboratory should establish prior agreements with a few selected laboratories to ensure that transfers of specimens are handled expeditiously. The transfer of specimens must be made in coordination with the MRO. If a laboratory chooses not to accept a specimen for retesting, it must contact the sender and make arrangements to forward the specimen to an alternate laboratory.

Issue 5: How does a laboratory interpret quantitative specimen validity results?

Comment: Truncating a quantitative value has been acceptable with "$\geq$", ">", and "<" decision points or cutoffs. However, truncating a quantitative value is not acceptable with "$\leq$" decision points or cutoffs. In "$\leq$" scenarios, truncating would change the result from acceptable to unacceptable (e.g., truncating a pH reading of 3.2 to 3 or a creatinine of 5.4 mg/dL to 5 mg/dL). Values from tests for creatinine ($\leq$5 mg/dL) or pH ($\leq$3) should contain one significant decimal place more than that specified in the stated decision point. For specific gravity ($\leq$ 1.001), the method must measure to the third (3^{rd}) decimal place. This will require refractometry because spectrophotometric and "paper/stick" procedures are not sensitive enough to accurately discriminate in that range.

Issue 6: What are the minimum quality control requirements for conducting a specimen validity test?

Comment: There should be at least one control in the "acceptable" range and one control in the "unacceptable" range analyzed with each batch of validity test specimens. Assays that have more than one decision point (i.e., creatinine, specific gravity, and pH) require more than one control in the unacceptable range: creatinine <20 mg/dL and $\leq$5 mg/dL; specific gravity $\geq$1.020, < 1.003, and $\leq$1.001; and pH $\leq$3 and $\geq$11. Controls should be prepared in an appropriate urine matrix and validated according to the laboratory's standard operating procedure (SOP) manual. In the case of pH controls, an appropriate buffer matrix may be used and the controls validated according to the laboratory's SOP manual. All controls must be validated prior to use.

Issue 7: Many laboratories have observed a significant increase in specimens which have a creatinine of $\leq$5 mg/dL, but have a specific gravity that is acceptable between 1.003 and 1.019. The specimens appear to be saline. These do not fit the definitions of "dilute" or "substituted" as stated in PD35. How are these specimens to be reported?

Comment: For specimens of this type, the laboratory should provide a "Specimen Unsuitable: Unable to Obtain Valid Drug Test Results" comment in block 7 of the CCF without reporting a "negative" drug test result.

Issue 8: Is more than one comment allowed when multiple adulterants are identified and the specimen is reported "Test Not Performed" or "Failed to Reconfirm"?

Comment: Although it is sufficient to provide only one comment listed in PD35 under "Test Not Performed" or "Failed to Reconfirm" to support an "adulterated" or "substituted" result, the laboratory may provide multiple comments if it has validity test results that require multiple comments.

Issue 9: Some manufacturers of immunoassay test kits have established an acceptable range for the pH of a specimen (e.g., 4.5 - 9). Can a laboratory reject a specimen as "unsuitable for testing" based on pH without determining whether it is adulterated (i.e., $\leq$ 3 or $\geq$ 11)?

Comment: Yes, a laboratory can reject specimens that do not meet its specimen acceptance criteria. Specimen rejection criteria are separate from specimen validity testing. Rejected specimens are reported as "Test Not Performed" with the comment "Specimen Unsuitable: Unable to Obtain Valid Drug Test Results." This comment is appropriate with a number of specimen rejection criteria, such as, the observation of foreign objects, unacceptable coloration, unacceptable viscosity, unacceptable odor, or when the pH indicates that the specimen is outside the recommended pH range established by the immunoassay test kit manufacturer. These criteria are separate from specimen validity testing and are not associated with the definitions of PD35.

Issue 10: Can a laboratory reject a specimen as "unsuitable for testing" based on pH instead of

reporting it as “adulterated” when pH is ≤3 or ≥11?

Comment: No, if a laboratory measures pH as a component of its specimen validity testing, it must adhere to the reporting criteria specified in PD 35. Moreover, the procedures utilized must be validated by the laboratory and follow the criteria outlined in paragraph 3. A. of this document.

Issue 11: Some laboratories indicate that assay performance is adversely affected when nitrite is present but <500 µg/mL. Can a laboratory report “unsuitable for testing” when nitrite is <500 µg/mL?

Comment: If the nitrite concentration is <500 µg/mL and the laboratory is unable to obtain a valid confirmatory test result, the laboratory may report “Test Not Performed.” In addition, the comment “Specimen Unsuitable: Cannot obtain valid drug test result” must be entered on the comment line indicating that it is the laboratory’s belief that the failure to obtain a valid confirmatory test result is caused solely by the presence of nitrite. However, if the laboratory is uncertain that nitrites are the cause for the failure to confirm because there may be an unidentified interfering substance/adulterant in the specimen, the laboratory **may** send the entire specimen to another certified laboratory (see issue 2 above).

Issue 12: Can “Adulterated” take precedence over “Reconfirmed the presence of ...” for a split specimen? For example, if a split specimen is positive for nitrite, is the split specimen reported “adulterated” even if the laboratory has reconfirmed an analyte that may or may not be affected by nitrite?

Comment: No. Since the primary (Bottle A) specimen was reported "positive" for a specific drug, laboratory B is always required to conduct the confirmatory test for that drug and to report it as "reconfirmed" if it reconfirms the analyte. If laboratory B is unable to reconfirm the presence of the drug, it must perform specimen validity testing to attempt to determine the reason for being unable to reconfirm the presence of the drug (PD35, Paragraph B.1.b).

Issue 13: The primary specimen (Bottle A) is reported substituted or adulterated. What must the laboratory do with Bottle B?

Comment: When a primary specimen (Bottle A) is reported adulterated or substituted, the laboratory must retain both Bottle A and Bottle B for a minimum of 12 months.

If you have any questions regarding these issues or comments, please contact my staff at (301) 443-6014.

/signed/

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